THE EFFECT OF ADRENALIN CHLORIDE AND TOAD VENOM ON THE BLOOD PRESSURE AND HEART RATE OF THE TROPICAL TOAD, BUFO MARINUS

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The tropical toad, *Bufo marinus*, secretes and stores in its skin glands a large amount of venom containing powerful adrenalin and digitalis-like substances without apparent harm to itself. Various workers have determined the pharmacological action and the minimum lethal dose of toad venom for animals other than the toad (Vulpian, 1854; Phisalix and Bertrand, 1893; Abel and Macht, 1912; Chen, Jensen, and Chen, 1931; Xavier, Vellard, and Vianna, 1931). Vulpian (1858) stated that the venom of the toad, which destroyed the irritability of the heart of the frog and of the salamander, had no action on the heart of the toad. Kobert (1887) found the vessels of the toad when perfused with toad venom to be less affected than those of the frog. Abel and Macht (1912) showed that both bufo-epinephrin and "bufagin," isolated from the venom, caused vasoconstriction when added to Locke solutions used to perfuse the blood vessels of *Bufo marinus*, and stated that the toad was relatively immune to "bufagin" but not to epinephrin. In the South African clawed toad, *Xenopus lævis*, Gunn (1930) found an adrenalin-like substance in the skin secretion which produced striking circulatory effects in the cat and the rabbit, but neither the skin secretion nor adrenalin had an effect on the circulatory system of *X. lævis*.

No reference in the literature could be found concerning the effect of adrenalin chloride or of toad venom on the blood pressure of the toad, nor even in any way concerning the measurement of blood pressure in this amphibian. Bieter and Scott (1929) found that an intravenous injection of 0.2 cc. of adrenalin chloride, 1 : 10,000, gave a rise of blood pressure lasting one hour and a quarter in the frog, *Rana pipiens*. The present report concerns the effect of intravenous injections of adrenalin chloride and of toad venom on blood pressure and heart rate in *B. marinus*. A determination of the minimum amounts of these substances necessary to produce a rise in blood pressure on intravenous injection is also reported.

Method

Specimens of *B. marinus* weighing from 215 grams to 590 grams were prepared by destroying the fore-brain through the *os frontoparietale* and pithing the spinal cord posterior to the second vertebra. The blood pressure was recorded by a mercury manometer of 3.5 mm. bore from a cannula in the *A. femoralis*. Sodium citrate, 7 per cent, was used as an anticoagulant and the cannula was wet with heparin before insertion. Injections were made with a Luer tuberculin syringe through a cannula in the *V. femoralis*. For threshold determinations the volume of fluid was generally not more than 0.2 cc. An intravenous injection of 0.5 cc. of amphibian Ringer's solution was without effect. Adrenalin chloride (Parke, Davis and Co.) was used in dilutions with Ringer's solution from 1 : 50,000 to 1 : 50,000,000. The venom was expressed on a weighed cover-glass from the large gland behind the ear, weighed, dissolved in Ringer's solution to known dilutions, and injected immediately. Laboratory temperatures varied from day to day between 27° C. and 29° C.

Results

The average systolic blood pressure in the femoral artery in nineteen toads about one-half hour after the destruction of the cerebral hemispheres and spinal cord and before injection was 33 mm. Hg, ranging from 22 mm. Hg to 56 mm. Hg. The heart rate was 76 per minute and the respiration 106 per minute. The pulse pressure varied from 4 mm. Hg to 12 mm. Hg. In two toads under ether anesthesia, with the nervous system intact, the blood pressure was 32 mm. Hg, the heart rate 64, and the respiration 106.

In twenty-four toads an intravenous injection of 0.2 cc. of adrenalin chloride 1 : 50,000, which was 0.8 μg. to 2 μg. per 100 grams (1 μg. equals 0.001 mg.), produced a rise in systolic blood pressure of 21 per cent to 155 per cent which lasted from 2 to 6 minutes (Fig. 1, *A*). Weaker doses such as 0.2 cc. of 1 : 200,000 regularly produced a pressor response; thus in one instance this dose was 0.33 μg. per 100 grams and gave a rise of 56 per cent. Subsequent doses were just as effective as first doses. The minimal effective dose of adrenalin chloride for a pressor response was found in nine toads to average 0.05 μg. per 100 grams with a range from 0.02 μg. to 0.09 μg. No depressor responses occurred even with doses as low as 0.002 μg.

While the effect of adrenalin on the heart rate in these preparations was not uniform but varied with the strength of dose and condition of the animal, there was generally a decrease. In fifteen toads doses from 0.2 cc. of 1 : 200,000 to 0.5 cc. of 1 : 50,000 (0.33 μg. to

2.5 μg. per 100 grams) gave a fall in heart rate of 6 per cent to 67 per cent associated with the rise of blood pressure. In three cases a rise of heart rate of less than 10 per cent occurred with a relatively large dose (1 μg. per 100 grams). In one case with a low heart rate of 44 per minute a dose of 2 μg. caused an increase to 56 per minute, while twenty minutes later a dose of 2.5 μg. caused a fall in heart rate from 68 to 57.

An intravenous injection of toad venom in Ringer's solution produced a rise in systolic pressure in ten toads in all doses from 160 μg.

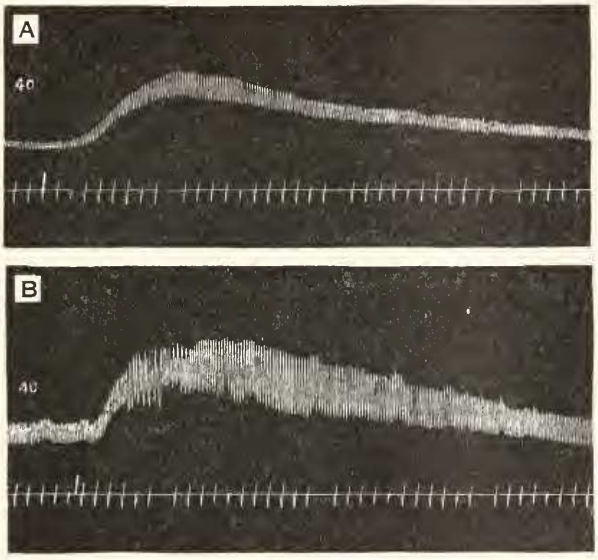

FIG. 1. *Bufo marinus*, 450 gram ♀, 29° C. Effect of intravenous injections on blood pressure and heart rate. *A*. Adrenalin chloride, 0.9 μg. per 100 grams. Rise in blood pressure, 145 per cent. Fall in heart rate, 9 per cent. Minimal pressor dose, 0.04 μg. *B*. Toad venom, 71 μg. per 100 grams. Rise in blood pressure, 123 per cent. Fall in heart rate, 22 per cent. Minimal pressor dose, 0.7 μg. Time in five-second intervals.

to 4 μg. somewhat in proportion to the amount of venom (Fig. 1, *B*). Thus, for example, 160 μg. gave a 292 per cent rise, while 15 μg. and 4 μg. gave 83 per cent and 33 per cent respectively. The minimum effective dose for a pressor response was found in five toads to average 0.5 μg. per 100 grams, with a range from 0.32 μg. to 0.71 μg. No depressor responses occurred with the small doses.

In nine toads the intravenous injection of the diluted toad venom caused a decrease (maximum 40 per cent) in the heart rate associated with the rise in pressure. In one case an increase occurred both with the venom and with adrenalin.

Discussion

The cardio-inhibitory effect of adrenalin associated with a rise of blood pressure suggests the presence of a reflex mechanism similar to that in mammals and certain lower vertebrates since in both the elasmobranch, *Squalus acanthias*, and the amphibian, *Necturus maculosus*, Lutz and Wyman (1932*a*, 1932*b*) have shown a reflex cardio-inhibitory mechanism stimulated by increased intravascular pressure.

In the reflex toad preparation the cardio-inhibition following the injection of venom must be due partly to the direct effect of "bufagin" (Abel and Macht, 1912) or cinobufotenine (Chen, Jensen, and Chen, 1931), although there was nothing in the response either of the blood pressure or of the heart to distinguish the effect of the venom from that of adrenalin alone.

Dragstedt, Wightman, and Huffman (1928) found the minimal effective pressor dose of adrenalin in the unanaesthetized dog to be less than from 0.2 cc. to 0.4 cc. of 1 to 1 million per kilogram per minute (0.02 μg. to 0.04 μg. per 100 grams). In rats, under urethane, Wyman and tum Suden (1932) generally obtained a detectable pressor response to adrenalin with 0.02 μg. per 100 grams, but in normal rats they found that 0.04 μg. per 100 grams was necessary to produce a visible vasoconstriction of the mesenteric vessels. These figures when compared with the pressor threshold for *B. marinus* to adrenalin (0.02 μg. to 0.09 μg. per 100 grams) indicate that this amphibian and the mammal are about equally sensitive to adrenalin. The total amount present at any time in the skin glands of the toad, the secretion of which yields about 5 per cent adrenalin according to Abel and Macht (1912), must be a great many times that present in the adrenal of the mammal (Sollman, 1932). According to Chen, Chen, and Jensen (1932) the adrenalin of the skin glands does not enter the blood stream although Phisalix and Bertrand (1893), using a questionable procedure, believed they found the toad venom in the blood in sufficient quantity for biological assay.

Since no depressor responses were obtained with adrenalin in doses as low as 0.002 μg. (one twenty-fifth the average pressor threshold), it appears that there are no vasodilators present in *B. marinus* sensitive to adrenalin.

Summary

1. Adrenalin chloride injected intravenously in *Bufo marinus* in doses of 0.33 μg. to 2 μg. per 100 grams produced a rise in systolic blood pressure of 21 per cent to 136 per cent. The average minimal effective dose was 0.05 μg. per 100 grams, ranging from 0.02 μg. to 0.09 μg.

2. Doses of 0.33 μg. to 2.5 μg. per 100 grams generally caused a decrease in heart rate of 6 per cent to 67 per cent associated with the rise of blood pressure.

3. Toad venom injected intravenously in *Bufo marinus* in doses of 4 μg. to 160 μg. per 100 grams produced a rise of systolic blood pressure of 33 per cent to 292 per cent. The average minimal effective dose was 0.5 μg. per 100 grams, ranging from 0.32 μg. to 0.71 μg.

4. The vasoconstrictor mechanism of *Bufo marinus* is one-tenth as sensitive to its venom as to adrenalin.

5. The response to adrenalin in *Bufo marinus* suggests the existence of a reflex cardio-inhibitory mechanism stimulated by increased intravascular pressure

6. No vasodilators are present in *Bufo marinus* sensitive to adrenalin chloride in doses down to one twenty-fifth of the minimal effective pressor dose.

7. The vasoconstrictor mechanisms of *Bufo marinus*, of the dog, and of the rat are about equally sensitive to adrenalin.

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